## REMARKS

In view of the following remarks, the Examiner is requested to allow claims 11, 13-15, 27 and 31, the only claims pending and under examination in this application.

Claims 1-10, 12, 16-26, 28-30 and 32-38 stand cancelled without prejudice against their reintroduction into this or one or more timely filed continuation, divisional or continuation-in-part applications.

Claim 11 is amended to specify that the insertion sequences flank a **"heterologous promoter and a single transcriptionally active gene"** and that said **"transcriptionally active gene is about 1000 bp or less from one of the P-element rocognized insertion sequences."** Support for this amendment is found at page 6, lines 11 to 25 of the specification, which reads:

> In the subject vectors, the pair of P-element encoded transposase recognized insertion sequences (i.e., P feet) flank at least one transcriptionally active gene that is in approximation to, i.e., is sufficiently close to or sufficiently proximal to, at least one of the P feet so as to provide for the desired genomic integration. By at least one is meant one or more, usually no more than five, and more usually no more than four, where the number of transcriptionally active genes in the vector is often one, two, or three, where only one of the transcriptionally active genes need be sufficiently proximal to the P foot. By in approximation to, i.e., sufficiently close or proximal to, is meant that the transcriptionally active gene is located at a distance from one of the flanking P-feet that is typically is less than about 7000 bp and often less than about 6,000; 5,000; 4,000; 3,000; or 2,000 bp, where in many embodiments the distance separating the transcriptionally active gene from the P foot does not exceed about 1,000 bp. In certain embodiments, the exogenous nucleic acid that is inserted into the genome of a whole animal in the subject methods, described in greater detail *infra*, is one of the transcriptionally active genes of the vectors.

Support may also be found throughout the specification, such as, for example at page 7, line 27 and page 16, line 1. As no new matter has been added by way of these amendments, entry thereof by the Examiner is respectfully requested.

### *Claim Rejections – 35 U.S.C. § 101*

Claims 11-15, 17, 18 and 27-38 have been rejected under 35 U.S.C. § 101 as directed to non-statutory subject matter. It is the position of the Examiner that the claims encompass any transgenic organism, the scope of which encompasses a human being, which is non-

statutory subject matter. The Examiner has suggested that the limitation "non-human" would be remedial. The rejection is now addressed by amendment and may be withdrawn.

***Double Patenting***

The Examiner provisionally rejects claims 12-15, 17-18 and 27-38 under 35 U.S.C. § 101 as allegedly claiming the same invention as claims 11, 13-15, 18, 27, 30-31 and 34 of co-pending Application No. 10/659,802. This rejection is respectfully traversed as applied to the currently pending claims.

The rejection is moot as applied to claims 12, 17-18, 28-30 and 32-38 as these claims stand cancelled. Although the Examiner has not included independent claim 11 in this rejection, the Examiner has rejected claims 13-15, which depend therefrom. Therefore, Applicants address the subject matter of claim 11, which involves inserting a heterologous promoter and a single transcriptionally active gene into the genome of a mouse or rat. In contrast, claim 11 of the '802 application is drawn to rodents generally and does not specify the presence of a heterologous promoter in front of a transcriptionally active gene. Accordingly, this rejection, which is based on the statutory type of double patenting, is improper as the allegedly conflicting claims are not coextensive in scope. Withdrawal is respectfully requested.

The Examiner also rejects claims 11-15, 17-18 and 27-38 under the judicially created doctrine of obvious-type double patent as allegedly unpatentable over claims 8-17 of U.S. Patent No. 6,475,798. This rejection is respectfully traversed as applied to the currently pending claims. The rejection is moot as to claims 12, 17-18, 28-30, and 32-38 as they stand cancelled.

The claims of the present application are directed to methods of introducing a heterologous promoter and a **single** transcriptionally active gene into the genome of a mouse or rat. In contrast, U.S. Patent No. 6,475,798 is directed to methods of inserting an exogenous nucleic acid into a non-insect target cell using a vector comprising a pair of P-element transposase sequences flanking at least **two** transcriptionally active genes.

The allegedly conflicting claims are not coextensive in scope. Therefore, Applicants respectfully request that this rejection be withdrawn.

***Claim Rejections – 35 U.S.C. § 112, first paragraph***

Claims 11-15, 17, 18 and 27-38 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification allegedly does not enable any person skilled in the art to make and use the claimed invention commensurate in scope with the claims. As applied to the currently pending claims, the rejection is respectfully traversed for at least the following reasons.

The Examiner acknowledges that enablement is present for a method of inserting an exogenous nucleic acid into the genome of a **mouse**, comprising:

> "...introducing into said mouse a P-element derived vector comprising a pair of P-element transposase recognized insertion sequences flanking at least one transcriptionally active gene that is at least 50 bp [*stet:in*] proximity to one of the P-element transposase recognized sequences and a transposase domain, and a method of inserting an exogenous nucleic acid into the genome of a mouse, wherein said method comprises introducing into said mouse a P-element derived vector comprising a pair of P-element trasposase recognized insertion sequences flanking at least one transcriptionally active gene that is at least 50 bp [*stet:in*] proximity to one of the P-element transposase recognized sequences, wherein said method further comprises inserting a second P-element vector comprising a transposase domain, and cells from said **mouse**." (Office Action at pages 4-5; emphasis added).

The law regarding enablement of inventions is clear: "[t]he test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosure in the patent coupled with information known in the art without undue experimentation."[1] In order to make a rejection, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. The Examiner must provide a

[1] *United States v. Telectronics, Inc.*, 8 USPQ 2d 1217, 1233 (Fed. Cir. 1988), *cert. denied*, 490 U.S. 1046 (1989). See also *Genentech, Inc. v. Novo Nordisk*, 42 USPQ 2d 1001 (Fed. Cir. 1997), *cert. denied*, 522 U.S. 963 (1997); *Scripps Clinic and Research Foundation v. Genentech, Inc.*, 18 USPQ 2d 1001 (Fed. Cir. 1991).

reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure.[2]

Further, the test for enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue.[3] The skilled artisan need not be able to predict in advance which modifications will result in successful practice of the claimed method in rats versus mice. Trial and error experimentation will readily provide this information. As noted by the Federal Circuit, trial and error experimentation is not necessarily undue.[4]

Applicants have amended the claims to specify that the claimed method is performed in a mouse. However, Applicants have also included the rat embodiment in this amendment, based on the disclosure in the Specification at page 19, lines 18-22, wherein it is set forth that male mice and rats were injected into their testis and these animals gave transgenic offspring.

Applicants maintain that the present application provides sufficient disclosure to enable the invention to the full scope of the pending claims with regard to mice and rats. Once transgenesis is demonstrated in one rodent species (mouse) using the P-element derived vectors from such a divergent and unrelated species (*Drosophila* fly of phylum Arthropoda), it is reasonable to conclude that the methods can be extrapolated to other rodents in a similar manner without undue experimentation. Rats are genetically and morphologically nearly identical to the mouse. Therefore, once the Applicants demonstrated the possibility of the described method with one species of rodent, it is reasonable to conclude that such methods can be used to generate transgenic rodents of different species using a vector that comprises a transposase recognized insertion sequence and an exogenous nucleic acid with a reasonable amount of experimentation.

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[2] *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993).
[3] *In re Angstadt*, 190 USPQ 214 (CCPA 1976).
[4] *See, e.g., In re Wands*, 858 F.2d 731 (Fed. Cir. 1988).

Accordingly, based on the disclosure in the specification, and the knowledge of those skilled in the art at the time of filing, the experimentation involved in selecting a rat or a mouse for use in practicing the claimed method does not rise to the level of "undue experimentation", as analyzed under the factors of *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988). Thus, Applicants respectfully request that the rejection under U.S.C. § 112, first paragraph, be withdrawn.

***Claim Rejections – 35 U.S.C. § 102***

Claims 11-15, 17, 18 and 27-38 have been rejected under 35 U.S.C. § 102(e) as allegedly anticipated by Fogarty et al. (U.S. Patent No. 6,475,798). As applied to the currently pending claims, the rejection is respectfully traversed for at least the reasons set forth below. In view of the amendments to the claims, this rejection may be withdrawn.

It is well established that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference."[5] Additionally, the identical invention must be shown in as complete detail as is contained in the claim.[6]

As noted above, the claims of the present application are directed to methods of inserting an exogenous nucleic acid into the genome of a mouse or rat, using a P-element derived vector that comprises a pair of P-element transposase recognized insertion sequences flanking a **single** transcriptionally active gene that comprises the exogenous nucleic acid.

In contrast, U.S. Patent No. 6,475,798 is directed to methods of inserting an exogenous nucleic acid into a non-insect target cell using a vector comprising a pair of P-element transposase sequences flanking at least **two** transcriptionally active genes. Since Fogarty et al. fails to teach a vector comprising a **single** transcriptionally active gene flanked by a P-

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5 *Verdegaal Bros. v. Union Oil Co. of California*, 2 USPQ 2d 1051, 1053 (Fed. Cir. 1987), *cert. denied*, 481 U.S. 1052 (1987). *See also, Scripps Clinic and Research Foundation v. Genentech, Inc.*, 18 USPQ 2d 1001 (Fed. Cir. 1991).
6 *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1566 (Fed. Cir. 1990).

element transposase recognized sequence, the cited reference fails to disclose each and every claimed of the present invention. Therefore, the Applicants respectfully request that this rejection be withdrawn.

***Claim Rejections – 35 U.S.C. § 102/103***

The Examiner rejects claims 11-15, 17, 18 and 27-38 under 35 U.S.C. § 102(b) as allegedly anticipated by, or in the alternative, under 35 U.S.C. § 103 as allegedly obvious in view of Khillan et al., *Dev. Bio.* 1985 109:247-250 (*hereinafter* "Khillan et al."). As applied to the currently pending claims, the rejection is respectfully traversed for at least the reasons set forth below.

*Novelty*

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.[7] Additionally, the identical invention must be shown in as complete detail as is contained in the claim.[8]

The claims specify that the **"transcriptionally active gene is separated from one of said P-element transposase recognized insertion sequences by a distance of about 1,000 bp or less"** and that the pair of P-element transposase recognized insertion sequences flank a **"heterologous promoter and a transcriptionally active gene."** Accordingly, Applicants present a genetically engineered construct which is spliced to include a spacer region and a heterologous promoter, such as SV40 or CMV. Therefore, the claims consist of a heterologous gene in place of the transposase.

Khillan et al. is completely silent as to these claim elements. Rather, the reference teaches a P-element insertion vector that consists simply of a P-element transpose coding sequence flanked by integration elements, or "P feet." The vector contains no promoter, and there is absolutely no space in between the insertion sequences and the transposase gene.

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[7] *Verdegaal Bros. v. Union Oil of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).
8 *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1566 (Fed. Cir. 1990).

(*See* Exhibit A). Moreover, the only gene that could possibly be transcriptionally active in this context is the P-element transposase itself.

Therefore, there cannot be a transcriptionally active gene that is separated **"from one of said P-element transposase recognized insertion sequences by a distance of about 1,000 bp or less."** As Khillan et al. fails to teach all the claimed elements, it is not effective as a reference under 35 U.S.C. § 102(b). Withdrawal is respectfully requested.

*Nonobviousness*

To establish a prima facie case of obviousness, three basic criteria must be met. One of these requires that the prior art reference, or references when combined, teach or suggest all the claim limitations.[9]

Here, independent claim 11 specifies that the **"transcriptionally active gene is separated from one of said P-element transposase recognized insertion sequences by a distance of about 1,000 bp or less"** and that the pair of P-element transposase recognized insertion sequences flank a **"heterologous promoter and a transcriptionally active gene."**

As shown above, Khillan et al. is completely silent with respect to these claim elements. Rather, the reference teaches a P-element insertion vector that consists simply of a P-element transpose coding sequence flanked by integration elements. The vector contains no promoter, and there is absolutely no space in between the insertion sequences and the transposase gene. (*See* Exhibit A). The only gene that could possibly be transcriptionally active in this context is the P-element transposase itself. The authors do not appreciate the advantages derived from placing a promoter element in front of the gene to be inserted, and in making this gene **"transcriptionally active."**

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9 *In re Royka*, 180 USPQ 580 (CCPA 1974).

Therefore, there cannot be a transcriptionally active gene that is separated **"from one of said P-element transposase recognized insertion sequences by a distance of about 1,000 bp or less."** As Khillan et al. fails to teach all the claimed elements, it is not effective as a reference under 35 U.S.C. § 103(a). Withdrawal is respectfully requested.

## CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number TOSK-007CIPCON.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: June 27, 2007

By: Gina Freschi
Gina C. Freschi
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Date: 6.27.07

By:
Bret E. Field
Registration No. 37,620

GCF:WHP

ENCLOSURE: EXHIBIT A P-ELEMENT ANATOMY FIGURE AND SPRADLING ET AL., *SCIENCE* 218:341-47(1982).

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# EXHIBIT A

## Figure 1

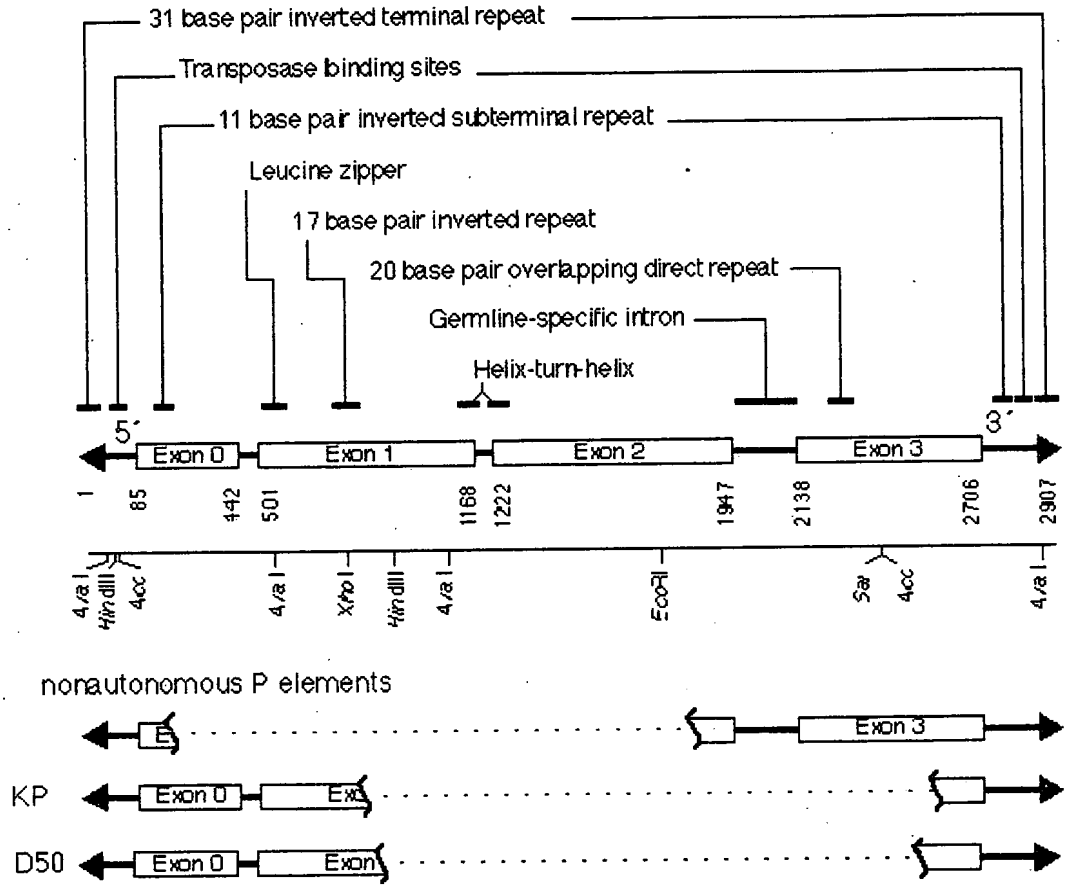


**Fig. 1. P element anatomy** (modified from Lindsley and Zimm 1992) : An autonomous P element is shown with some of its sequence features and a restriction map. Three examples of nonautonomous P elements, including the Type II repressor-making elements, KP and D50. For the complete P element sequence, see the Genbank entry provided by O'Hare and Rubin (1983).

Back to P Element Index

Site admin: - Bill Engels, .... 16 March 1996

# Transposition of Cloned P Elements into *Drosophila* Germ Line Chromosomes

Allan C. Spradling and Gerald M. Rubin

*Summary.* Recombinant DNA carrying the 3-kilobase P transposable element was injected into *Drosophila* embryos of a strain that lacked such elements. Under optimum conditions, half of the surviving embryos showed evidence of P element–induced mutations in a fraction of their progeny. Direct analysis of the DNA of strains derived from such flies showed them to contain from one to five intact 3-kilobase P elements located at a wide variety of chromosomal sites. DNA sequences located outside the P element on the injected DNA were not transferred. Thus P elements can efficiently and selectively transpose from extrachromosomal DNA to the DNA of germ line chromosomes in *Drosophila* embryos. These observations provide the basis for efficient DNA-mediated gene transfer in *Drosophila*.

The ability to transfer exogenous genetic information into living cells has proved to be a valuable tool in the study of the structure, function, and regulation of genes in unicellular organisms such as bacteria and yeast. The development of efficient and reproducible procedures for DNA-mediated gene transfer in metazoans, however, has lagged far behind largely because of the lack of appropriate vectors.

Potentially suitable vectors for gene transfer occur in nature in the form of viruses and transposable elements. Transposable elements are DNA segments which, as discrete units, are capable of changing their positions within the genome of a cell (*1*). In bacteria, these elements have been shown to also transpose from extrachromosomal DNA such as plasmids, into chromosomal sites. No eukaryotic transposon with this property has yet been described. If such a eukaryotic transposable element could be identified it might then serve as an efficient transformation vector. Cloned DNA containing the element could be introduced into cells and transpositions from this exogenous DNA into the host chromosomes might take place at high frequency.

Several classes of transposable elements have been identified in the genome of the fruit fly, *Drosophila melanogaster* (*2*). The properties of one class, the P elements (*3*), recommend it as a possible gene transfer vector: these elements transpose at very high rates when certain genetic criteria are met. The P factors, which are probably a subset of the P element family, are the primary causal agents in a syndrome of correlated genetic traits, known as P-M hybrid dysgenesis (*3*, *4*) that occurs among the progeny of matings between certain *Drosophila* strains. These traits, which are limited primarily to the germ line, include high rates of mutation, frequent chromosomal aberrations and, in extreme cases, the failure to produce any mature germ line cells. Dysgenesis occurs when males of a P (paternally contributing) strain are mated with females of an M (maternally contributing) strain, but usually not when the reciprocal cross is performed.

P strains are distinguished genetically from M strains by virtue of multiple genetic elements, the P factors, which are dispersed over all the major chromosome arms. The P factors do not produce dysgenesis within P strains, but do so only when placed in the maternally derived background of an M strain [M cytotype (*5*)]. Moreover, the stability of mutations arising in dysgenic flies appears to be under the same control system as all other manifestations of hybrid dysgenesis: they do not revert when maintained in a P strain (P cytotype), but they may revert at high frequencies when placed in the M cytotype.

These and other observations led to the proposal that hybrid dysgenesis results from the action of a family of transposable elements, the P factors. In its simplest form, this hypothesis states that P factors are present in P strains, where their transposition is repressed, and are absent from M strains. When chromosomes carrying P factors are placed in the M cytotype, it is proposed that these elements become derepressed and transpose at high rates. Among other effects, P factors would then induce mutations by inserting into and disrupting genetic loci. Such dysgenesis-induced mutations would be expected to be stable in the P cytotype, where P factor transposition is repressed, but unstable in the M cytotype where they could revert by excision of the P factor.

Recent molecular and genetic data strongly support the basic features of this model. Several mutations arising in dysgenic crosses between P and M strains have been shown to be due to the insertion of members of a single family of transposable elements, named P elements, which are found in the chromosomes of P strains but are absent from M strains (*3*). Although the P element insertions that cause these mutations are homologous in sequence, they are heterogeneous in size, ranging from 0.5 to 1.6 kilobases (kb). The small size and heterogeneity of the P elements suggest that they would be incapable of encoding the genetic functions attributed to the P factor. Thus, the transposition of the small P elements would require the presence elsewhere in the genome of the P factor, which is proposed to encode a P element–specific transposase. (We use the term "transposase" although the biochemical mechanism of this *trans*-acting product is unknown.) Genetic data supporting this view of the P elements as a two-element system have been obtained [see (*6*) and below]. Such a transposable element system would then be analogous to two element systems in prokaryotes (*7*) and in maize (*8*).

A candidate for the P factor has been isolated (*9*). This 3-kb P element is present in several nearly identical copies in the genomes of P strains. DNA sequence analysis revealed that the smaller heterogeneous P elements could have been derived from this larger element by internal deletions (*9*). Both the 3-kb P element and the smaller elements contain the same 31 base pair (bp) perfect inverted repeat at their termini; it is likely that this DNA sequence is the site of action of the putative P element–specific transposase. Moreover, the presence of three

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0036-8075/82/1022-0341$01.00/0 

long open reading frames for translation within the 3-kb element suggests that it may have the capacity to encode the transposase and regulatory product (or products) expected for the P factor.

If the 3-kb element is indeed the P factor, it should be capable of providing the functions required both for its own transposition and for transposition of the smaller P elements when placed in M cytotype. Consequently, we tested the ability of a cloned P element to transpose after introduction into embryonic cells. We now demonstrate that the 3-kb element can provide the functions required for its own transposition from exogenously introduced DNA into the chromosomes of germ line precursor cells. These results provide the basis for the development of vectors for efficient DNA-mediated gene transfer in *Drosophila*.

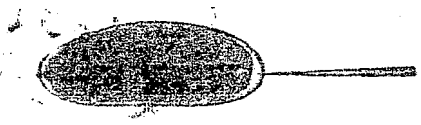

Fig. 1. Injection of DNA into a *Drosophila* embryo. The length of the embryo is approximately 0.5 mm. The tip of the needle is located in the posterior pole of the embryo, the site of germ cell formation.

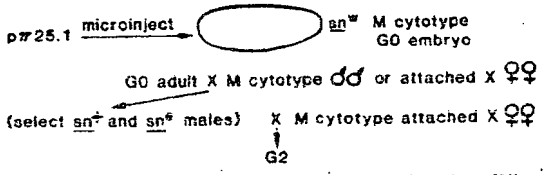


Fig. 2. Protocol for P element transformation. DNA is microinjected into an embryo from the $sn^w$ M strain prior to pole cell formation [see (*10*) for details]. After development is completed, the adult (G0) is mated to $sn^w$ M males or attached-X females. When males are mated at attached-X bearing females, the only male progeny carry the paternal X chromosome. Therefore regardless of the sex of the injected embryo, their male progeny (G1 generation) will contain an X chromosome which was present in the germ cells of the injected embryo. To avoid introducing a chromosomal source of P elements the strains used for mating to the G0 adults totally lacked P elements in their genomes and were therefore of the M cytotype. As a control for possible contamination, the chromosomes of the host strain were also homozygous for recessive mutations. The X chromosome was marked with *yellow*, chromosome 2 with *brown*, and chromosome 3 with *scarlet*. Flies homozygous for all three mutations have yellow body color and white eyes. Any contamination of the host stock would have been revealed by the loss of these recessive phenotypes.

Table 1. Microinjection of pπ25.1 DNA into $sn^w$(M) embryos.

| pπ25.1 DNA (μg/ml) | Injected (No.) | Hatched (No.) | Eclosed (No.) | Fertile (No.) | Mutable | |
|---|---|---|---|---|---|---|
| | | | | | No. | Percent† |
| 0* | 183 | 72 | 48 | 34 | 0 | 0 |
| 0.1 | 184 | 57 | 34 | 30 | 0 | 0 |
| 10 | 397 | 93 | 60 | 43 | 2 | 5 |
| 100 | 363 | 110 | 77 | 56 | 27 | 48 |
| 1000 | 522 | 173 | 123 | 100 | 2 | 2 |

*Embryos in this experiment received DNA lacking 3-kb P elements at 1000 μg/ml. †Percent of fertile adults which were mutable.

Table 2. Phenotype of male progeny of injected embryos displaying germ line mutability. The *sn* phenotype of the G1 male progeny of 31 mutable G0 adults is shown. Embryos 101 and 102 received DNA at 10 μg/ml; embryos 201 to 227 received 100 μg/ml; and embryos 301 and 302 received 1000 μg/ml.

| Embryo | Progeny phenotype (*20*) | | | Embryo | Progeny phenotype (*20*) | | |
|---|---|---|---|---|---|---|---|
| | $sn^+$ | $sn^w$ | $sn^e$ | | $sn^+$ | $sn^w$ | $sn^e$ |
| 101 | 5 | 34 | | 215 | 7 | 66 | |
| 102 | 6 | 81 | | 216 | 5 | 38 | |
| 201 | 3 | 31 | 6 | 217 | 2 | 21 | |
| 202 | 3 | 38 | 3 | 218 | | 57 | 4 |
| 203 | 1 | 114 | | 219 | 6 | 36 | 3 |
| 204 | 1 | 33 | | 220 | 3 | 59 | |
| 205 | 3 | 70 | 11 | 221 | 4 | 31 | 1 |
| 206 | | 117 | 1 | 222 | 2 | 42 | |
| 207 | 1 | 67 | 5 | 223 | 1 | 48 | 1 |
| 208 | 1 | 119 | 1 | 224 | 1 | 43 | |
| 209 | 1 | 45 | 1 | 225 | | 30 | 4 |
| 210 | 2 | 63 | 4 | 226 | 5 | 42 | |
| 211 | 1 | 42 | 2 | 227 | | 44 | 1 |
| 212 | | 5 | 1 | 301 | | 17 | 1 |
| 213 | 2 | 32 | 22 | 302 | 2 | 159 | |
| 214 | 10 | 53 | 3 | | | | |

### Experimental Design

Our approach is based on mimicking the events that take place during a dysgenic cross between P and M strains. In such a cross, P factors enter the M cytotype egg with the sperm. These P factors and other P elements are thereby induced to transpose at high frequency. We reasoned that an analogous situation might occur if DNA containing the 3-kb P element were introduced by microinjection into an M cytotype embryo shortly after fertilization. Early in *Drosophila* embryonic development, the nuclei of the embryo go through several rounds of synchronous division before they are compartmentalized into cells. At completion of the ninth nuclear division about a dozen nuclei migrate to the posterior pole of the egg where they form the germ line precursor cells, or pole cells. To maximize the chance that the injected plasmid DNA enters the germ line precursor cells, we introduced DNA, by microinjection, into the posterior end of the egg (*10*) just before pole cell formation (Fig. 1).

Because of the multiplicity of germ line precursor cells, P element DNA transferred into the genome of a single pole cell will be inherited by only a fraction of the progeny of that embryo. Therefore all the progeny of the injected embryo would have to be tested individually for the presence of the injected DNA sequences. Since this entails screening large numbers of flies, an assay based on a change in a visible phenotype, rather than a biochemical assay for the physical presence of the DNA sequence, was devised.

Our assay was based on the properties of an unusual allele of the *singed* (*sn*) locus, an X-linked genetic locus that controls the morphology of the bristles and hairs on the cuticle of the adult fly. This allele, *singed-weak* ($sn^w$), arose in an individual undergoing hybrid dysgenesis (*11*), and available evidence suggests that the mutant phenotype results from the insertion of one or two small P elements into the *sn* locus. In a fly strain that carries only these small P elements

at *sn* and no P factors in its genome, the $sn^w$ allele is phenotypically stable even though the cytotype is M (*6*). We refer to this strain as the $sn^w$ M strain. When females from this strain are mated to P males, however, the $sn^w$ allele mutates at very high rates in the germ line of their dysgenic progeny. These mutations become visible in the progeny of these dysgenic individuals; only about half display the parental $sn^w$ phenotype, the others are divided between a more severe allele, *singed-extreme* ($sn^e$), and wild type ($sn^+$). This hypermutability depends on the introduction of P factors into the strain; the same $sn^w$ mutation is completely stable when maintained in the $sn^w$ M strain. Thus the induction of $sn^w$ mutability provides a convenient genetic assay for the presence of functional P factors. The most likely explanation for the behavior of the $sn^w$ mutation is that the P elements at the *sn* locus are incapable of catalyzing their own transposition but are able to respond to transposase produced by P factors.

Our experimental protocol for detecting P element transposition is diagrammed in Fig. 2. Host embryos from the $sn^w$ M strain were injected with DNA of the plasmid pπ25.1, which contains a 3-kb P element and about 1.8 kb of flanking *Drosophila* DNA, cloned in the *Escherichia coli* plasmid vector pBR322. Adult flies derived from injected embryos (G0 males and females) were mated to attached-X females, or to $sn^w$ M males. The bristles of male progeny of these crosses (G1 males) were examined to determine which allele of *sn* they carried.

The G1 males displaying either the $sn^+$ or $sn^e$ phenotype provide evidence that the 3-kb P element carried on the injected plasmid DNA was capable of directing the synthesis of a transposase that destabilized the small P elements resident at the $sn^w$ locus in the germ line of the G0 host. (Since hybrid dysgenesis does not destabilize $sn^w$ in somatic tissues, the phenotype of the G0 flies themselves cannot be used to assay for transposase activity.) Such a transposase might also be able to catalyze the transposition of the 3-kb element from the injected plasmid DNA into the chromosomes of the injected egg. The destabilization of the P elements at *sn* and the transposition of the 3-kb element are separate events. Since they would require the same transposase function, however, we would expect them to be highly correlated. If a 3-kb P element became integrated and its putative transposase gene remained functional, the *singed* locus might continue to be unstable in subsequent generations. Thus by examining the *singed* phenotypes of the G2 males we could genetically assay for the heritability of the injected P element. The results of these genetic assays could then be confirmed by direct physical measurements of the location and structure of any P element now resident in the genome.

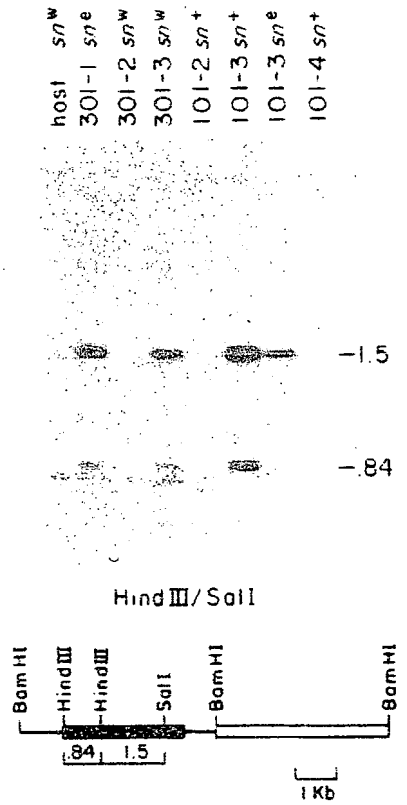


Fig. 3. P element DNA sequences in stable and mutable G1 lines. DNA from 200 to 300 males of each G1 line derived from embryos 301 and 101 was prepared. (DNA was prepared separately from the $sn^+$ and $sn^e$ males of the 101-3 mutable line.) Each DNA (2 μg) was digested with Hind III plus Sal I, fractionated by electrophoresis on a 1.0 percent gel, partially depurinated, and transferred to nitrocellulose paper. The probe consisted of equal amounts of the 0.84-kb Hind III fragment and the 1.5-kb Hind III–Sal I fragment of pπ25.1 indicated in the diagram, which had been labeled with $^{32}P$ by nick translation. A linear map representing the circular plasmid pπ25.1 is shown. The solid bar indicates the position of the 3-kb P element. The thin line represents flanking *Drosophila* genomic DNA sequences from cytogenetic locus 17C and the open bar depicts pBR322 vector sequences. Bands of about 0.8 kb and 0.6 kb were labeled by the probes in DNA from the $sn^w$ host strain. They are presumed to derive from the P elements present in the vicinity of *singed*. One of these bands (0.6 kb) was absent in all the lines tested that had undergone mutation to $sn^e$. This correlation is consistent with the idea that $sn^w$ mutability is the result of changes in the arrangement of P element sequences. A second band (0.8 kb) was present in all lines tested except 101-3. The change in this band did not correlate with a changed *singed* phenotype.

### Injected P Elements Induce $sn^w$ Mutability

The results of injecting a constant volume of pπ25.1 DNA at various concentrations into embryos according to the scheme shown in Fig. 2 are summarized in Table 1. The approximate percentage of the injected embryos which hatched as larvae, eclosed from the pupal case, and which were fertile did not vary significantly with the concentration of the injected DNA (*12*). However, the fraction of G0 organisms showing germ line mutations at the *singed* locus was highly dependent on the concentration of injected plasmid DNA. When embryos were mock-injected, or injected with a dilute solution of DNA (0.1 microgram per milliliter) no mutable flies were obtained. At higher concentrations, however, injected flies producing $sn^+$ or $sn^e$ G1 progeny were observed. Injection of the P factor–containing plasmid at a concentration of 100 μg/ml produced the highest frequency (48 percent) of mutable individuals (*12*).

The induction of *singed* mutability by the injection of the pπ25.1 plasmid suggested that the injected DNA produced transposase that acted at the *singed* locus in the chromosomes of one or more germ line cells. Table 2 illustrates that the appearance of $sn^+$ or $sn^e$ G1 offspring from the mutable parent sometimes occurred in clusters. This argues that the $sn^w$ mutations induced by the injected DNA can take place premeiotically, as they do under the conditions of hybrid dysgenesis studied previously (*11*), and as would be expected if transposase is produced relatively early in germ line development. The data are also compatible with the occurrence of some $sn^w$ mutations during meiotic or postmeiotic stages.

To determine whether the ability to induce $sn^w$ hypermutability was inherited by the progeny of G0 mutable flies, $sn^+$, $sn^e$, and some $sn^w$ male G1 progeny of each of the 31 mutable G0 individuals were mated to attached-X M females (Fig. 2). Table 3 summarizes the *sn* phenotypes of the G2 males generated from these crosses. In the majority of cases at least one of the G1 progeny of each mutable fly also demonstrated germ line mutations at the *sn* locus. Thus, levels of transposase adequate to cause $sn^w$ mutability were found not only in the germ cells of the injected embryo but in the germ cells of some of its progeny. In all cases tested, $sn^w$ mutability continued to be observed in subsequent generations when males from a G1 mutable stock were mated to attached-X M females.

In this respect, therefore, these strains behave as if they carried chromosomal P factors.

To determine whether this genetic behavior was indeed the result of the integration of one or more P factors from the injected DNA into the chromosomes of the host germ line, the DNA from several of the mutable strains was analyzed (Fig. 3). Two of the G1 male progeny of the mutable G0 embryo 301 (301-1 and 301-3) showed continued mutability while a sibling male (301-2) was phenotypically stable (see Table 3). Likewise, one G1 male progeny of G0 embryo 101 was mutable (101-3) and two were stable (101-2 and 101-4). DNA was prepared from the $sn^w$ M host strain and from the G2 male progeny of these six G1 males. The presence of one or more complete P elements was assayed by digesting the DNA's with Hind III and Sal I, transferring the digests to nitrocellulose paper after agarose gel electrophoresis, and hybridizing with two subcloned fragments internal to the P element (Fig. 3). The absence of 0.84- and 1.5-kb bands in host DNA confirmed that no complete P elements are present in the $sn^w$ strain. Neither line 301-2, 101-2, nor 101-4, which were phenotypically stable, showed the presence of any bands not observed in host DNA. However, DNA from the unstable lines 301-1, 301-3, and 101-3 showed strong bands of hybridization at 0.84 kb and 1.5 kb, consistent with the presence of one or several complete P factors. This correlation between continued mutability and the presence of new P element sequences was verified by similar experiments on the progeny of eight other injected embryos (*13*).

Table 3. Induction of *sn* mutability by individual male G1 progeny of mutable G0 adults. The *sn* phenotypes of G2 progeny males derived from individual G1 males (see Table 2) by crossing them to attached-X females are shown. G1 male progeny of the same G0 injected fly are numbered consecutively using the same identifying number as in Table 2. Abbreviations: e, $sn^e$; w, $sn^w$; +, $sn^+$.

| Individual | Progeny phenotype | | | | Individual | Progeny phenotype | | | | Individual | Progeny phenotype | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *sn* Phenotype | $sn^+$ | $sn^w$ | $sn^e$ | | *sn* Phenotype | $sn^+$ | $sn^w$ | $sn^e$ | | *sn* Phenotype | $sn^+$ | $sn^w$ | $sn^e$ |
| 101-1 | + | 110 | | | | | | | | | | | | |
| 101-2 | + | 160 | | | 205-16 | e | | | 73 | 215-3 | + | 104 | | |
| 101-3 | + | 209 | | 16 | 205-17 | e | 3 | | 87 | 215-4 | + | 96 | | |
| 101-4 | + | 54 | | | 206-1 | e | | | 133 | 215-5 | + | 98 | | |
| 101-5 | + | 124 | | | 207-1 | e | | | 68 | 215-6 | + | 63 | | |
| 102-1 | + | 107 | | 1 | 207-2 | + | 132 | | 1 | 215-7 | + | 32 | | |
| 102-2 | + | 39 | | | 207-3 | e | 6 | | 99 | 216-1 | + | 88 | | |
| 102-3 | + | 140 | | | 207-4 | e | | | 108 | 216-2 | + | 88 | | 1 |
| 102-4 | + | 144 | | | 207-5 | e | | | 108 | 216-3 | + | 130 | | |
| 102-5 | + | 178 | | | 208-1 | e | 1 | | 122 | 216-4 | + | 67 | | |
| 201-1 | + | 93 | | | 208-2 | + | 128 | | | 217-1 | + | 74 | | |
| 201-2 | e | | | 100 | 209-1 | + | 132 | 3 | | 217-2 | + | 60 | | |
| 201-3 | e | | | 107 | 209-2 | w | 4 | 43 | 2 | 218-1 | e | | | 91 |
| 201-4 | + | 149 | | | 210-1 | e | | | 141 | 218-2 | e | 2 | | 75 |
| 201-5 | e | | 3 | 105 | 210-2 | e | | 1 | 61 | 218-3 | e | | | 75 |
| 201-6 | e | | | 97 | 210-3 | w | 8 | 110 | 10 | 218-4 | e | | | 121 |
| 201-7 | e | | 1 | 93 | 210-4 | + | 139 | | | 219-1 | e | | | 104 |
| 201-8 | e | | 1 | 98 | 210-5 | w | | 46 | | 219-2 | e | | | 101 |
| 201-9 | + | 102 | 1 | | 210-6 | e | | | 73 | 219-3 | e | | | 95 |
| 201-10 | w | 29 | 198 | 4 | 210-7 | e | | | 66 | 219-4 | + | 88 | | |
| 201-11 | w | | 118 | | 210-8 | + | 87 | | 1 | 219-5 | + | 77 | | |
| 201-12 | w | 1 | 117 | 1 | 211-1 | + | 3 | | | 219-6 | + | 67 | | |
| 201-13 | w | | 80 | | 211-2 | e | 3 | | 92 | 219-7 | + | 106 | | |
| 201-14 | w | | 64 | | 211-3 | e | | | 80 | 2.9-8 | + | 48 | | |
| 201-15 | w | | 124 | | 212-1 | e | | | 64 | 219-9 | + | 106 | | |
| 201-16 | w | 3 | 66 | 11 | 212-2 | w | | 77 | | 220-1 | + | 155 | | |
| 202-1 | + | 45 | | | 212-3 | w | | 76 | | 220-2 | + | 68 | | |
| 202-2 | + | 58 | | | 213-1 | e | | | 106 | 220-3 | + | 95 | | |
| 202-3 | e | | | 75 | 213-2 | e | | | 72 | 221-1 | + | 131 | | |
| 202-4 | e | | | 125 | 213-3 | e | | | 60 | 221-2 | + | 95 | | |
| 202-5 | w | 31 | 57 | 6 | 213-4 | e | | | 41 | 221-3 | + | 62 | | 2 |
| 202-6 | w | 20 | 94 | 1 | 213-5 | e | 2 | | 77 | 221-4 | + | 125 | | 1 |
| 202-7 | w | 7 | 87 | 11 | 213-6 | w | | 82 | | 222-1 | + | 111 | | |
| 202-8 | + | 113 | 1 | | 213-7 | w | | 77 | | 222-2 | + | 102 | | |
| 203-1 | + | 84 | | | 213-8 | w | | 90 | | 223-1 | e | | | 116 |
| 204-1 | + | 61 | 66 | 1 | 213-9 | w | 24 | 66 | 5 | 224-1 | + | 142 | | |
| 205-1 | e | 2 | | 136 | 213-10 | e | | | 112 | 225-1 | e | | | 99 |
| 205-2 | w | | 131 | 6 | 213-11 | e | 3 | | 73 | 225-2 | e | 1 | | 72 |
| 205-3 | w | 14 | 84 | 19 | 213-12 | + | 71 | | | 225-3 | e | | | 106 |
| 205-4 | + | 72 | | | 213-13 | + | 24 | | | 225-4 | e | | | 12 |
| 205-5 | + | 129 | | | 214-1 | e | 3 | | 61 | 226-1 | + | 85 | | |
| 205-6 | w | | 126 | | 214-2 | e | 6 | | 64 | 226-2 | + | 112 | | |
| 205-7 | e | 1 | | 120 | 214-3 | + | 96 | | 2 | 226-3 | + | 97 | | |
| 205-8 | e | 3 | 1 | 101 | 214-4 | + | 82 | | | 226-4 | + | 92 | | |
| 205-9 | e | 2 | | 115 | 214-5 | w | | 87 | | 226-5 | + | 67 | | |
| 205-10 | e | | | 113 | 214-6 | w | | 58 | | 227-1 | e | | | 94 |
| 205-11 | e | | | 113 | 214-7 | w | | 71 | | 301-1 | e | 5 | | 329 |
| 205-12 | + | 132 | | | 214-8 | + | 85 | | 2 | 301-2 | w | | 100 | |
| 205-13 | + | 125 | | | 214-9 | + | 73 | | | 301-3 | w | 12 | 227 | 13 |
| 205-14 | e | 4 | | 127 | 215-1 | + | 73 | | | 301-4 | w | | 287 | |
| 205-15 | e | 1 | | 88 | 215-2 | + | 115 | | | 302-1 | + | 100 | 8 | |

### Mutable Lines Contain Chromosomally Integrated P Elements

To see whether the additional P element sequences in the unstable lines were present at specific chromosomal loci, polytene salivary gland chromosomes were prepared from the 301 strains and hybridized in situ with pπ25.1 sequences. Only two sites were labeled in chromosomes from the host $sn^w$ strain, or in the stable G1 lines (Fig. 4). Grains were detected at 17C (*14*) because the *Drosophila* DNA flanking the 3-kb P factor in pπ25.1 is derived from this region. The vicinity of the *singed* gene, 7D, was also labeled, because of the presence of small P element (or elements) associated with the $sn^w$ allele. Both of the unstable lines 301-1 and 301-3 contained an additional strong site of hybridization on the X chromosome at 12F (Fig. 4). The continued $sn^w$ instability and the presence of internal P factor restriction fragments in the 301-1 and 301-3 strains are therefore most simply explained by the integration of P factor sequences derived from the injected pπ25.1 DNA into the X chromosome at chromosomal site 12F. Figure 5 summarizes similar studies on chromosomes from progeny of 23 of the 31 mutable embryos. In all the cases examined, strains showing continued $sn^w$ mutability in the germ line of the G1 generation contained from one to five sites of in situ hybridization not present in the $sn^w$ host (*15*). No new sites of labeling were detected in 12 stable strains examined. The sites in the unstable strains were widely distributed on all the chromosome arms. P factors are known to be capable of inserting at a wide variety of sites (*16*). The number of grains at the new sites was consistent with the insertion of one complete element per site, usually about twice the number present at 17C on the same slide. However, in a few cases the hybridization at a chromosomal site was less than expected relative to 17C. Lightly labeled sites may be the location of small P elements resulting from transposition of elements resident at $sn^w$ or deriving from a 3-kb P element by a new deletion event.

### Integration Occurs by Transposition

If the integration of P element sequences in the mutable lines occurs by transposition, genomic DNA from these lines should contain the entire 3-kb P element sequence but lack the flanking *Drosophila* and pBR322 vector sequences in pπ25.1. These expectations were verified by hybridizing DNA's from several mutable strains with specific probes containing pπ25.1 sequences (Fig. 6). DNA from the $sn^w$ host and from five independently derived $sn^w$ mutable lines was digested with Hind III and Sal I. After separation on a 1.0 percent agarose gel, duplicate filters were prepared which contained each of the six DNA's. One of the filters was hybridized with $^{32}$P-labeled pπ25.1 DNA (Fig. 6A) while the second (Fig. 6B) was hybridized to a pS25.1, a plasmid probe that contained the same chromosomal Bam HI fragment as pπ25.1 but lacked the inserted 3-kb P element. [This Bam

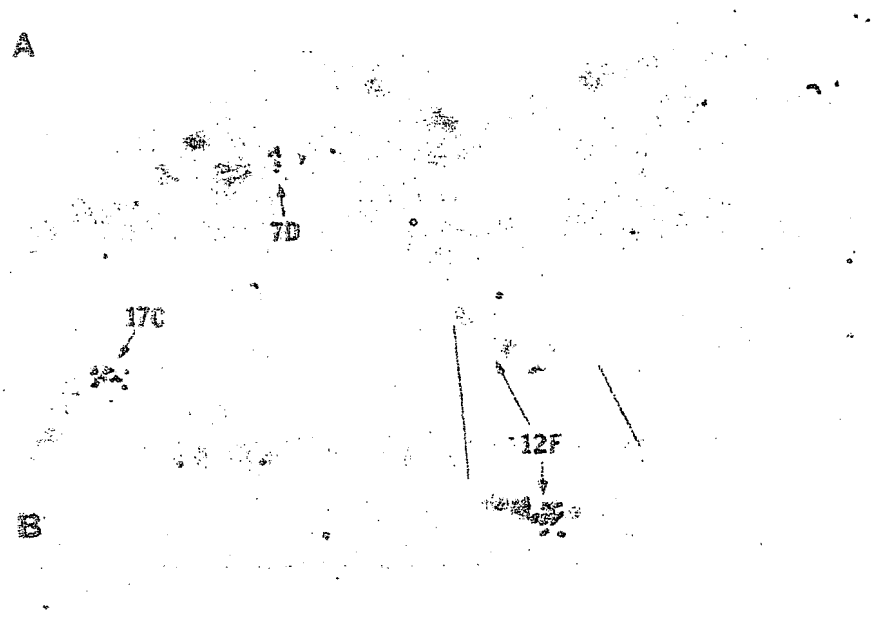


Fig. 4. Chromosomal sites complementary to pπ25.1 DNA sequences. Polytene salivary gland chromosomes were prepared from larvae of the $sn^w$ M strain (A), and the 301-2 G1 line (B). In situ hybridization was carried out (*21*) with $^3$H-labeled RNA complementary to pπ25.1 DNA as probe. Only two sites were labeled in the host $sn^w$ M strain chromosomes: the site of the unique chromosomal sequences flanking the 3-kb P element in pπ25.1 [17C (*14*)], and the site of the $sn^w$ locus (7D), which contains small P elements complementary to pπ25.1. Besides these two sites, one additional site (12F) was labeled in 301-2 chromosomes.

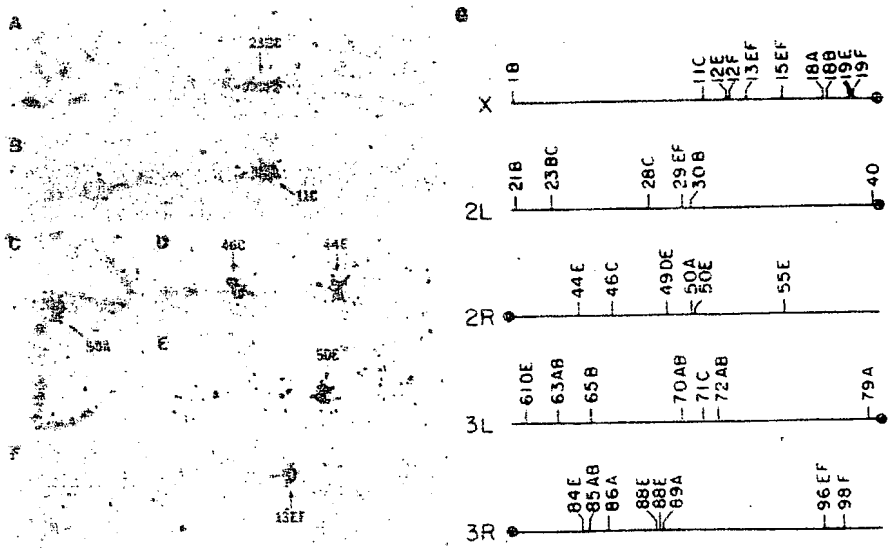


Fig. 5. Sites of P element integration. Polytene chromosomes from mutable G2 lines were hybridized in situ with pπ25.1 complementary RNA sequences as described in Fig. 4. Examples of the sites of hybridization observed are shown in (A to F). The chromosomal distribution of all 38 sites observed is represented in panel G. Each of the five major chromosomal arms is indicated, and the positions of centromeres are shown by filled circles [see (*14*)]. The karyotypes of these salivary gland cells were usually normal, but chromosome rearrangements were observed in some lines (*19*).

fragment was cloned from the Canton S strain, which does not have a P element at 17C (9).] The structure of these probes is shown in Fig. 6. Multiple Hind III–Sal I fragments were labeled by pπ25.1 in each of the mutable strain DNA's, including the expected 0.84-kb and 1.5-kb fragments internal to the P element (Fig. 6A). The top band seen in Fig. 6, A and B, must correspond to the chromosomal sequences surrounding the P element in pπ25.1 since it was the only fragment labeled by the pS25.1 probe in DNA from the host strain. Since no additional fragments were labeled by pS25.1 in DNA from any of the mutable strains, the additional bands labeled by pπ25.1 must contain only P element sequences. The presence of as little as 100 bp of flanking *Drosophila* DNA or pBR322 sequences would probably have been detected in these experiments. These results are also inconsistent with the presence of free pπ25.1 plasmid DNA in these strains. Further evidence for the presence of one or more complete 3-kb P elements in the mutable lines was provided by analyzing the same DNA's digested with Ava II. These digests were probed with the 0.84-kb Hind III and 1.5-kb Hind III–Sal I fragments of pπ25.1. All the Ava II fragments present in the 3-kb P element were labeled in each of the mutable lines (Fig. 6C). Together these Ava II fragments comprise the entire element except for 22 bp within each terminal repeat (9). These experiments suggest that the entire P element, but no other DNA sequence from pπ25.1, is present in each of the mutable lines.

## Concluding Remarks

These experiments demonstrate that P element sequences tranpose with high efficiency from plasmid DNA into the chromosomes of germ line cells at diverse sites after injection of the DNA into early *Drosophila* embryos. At least one of the injected P elements in each of the mutable lines is functional as evidenced by the induction of $sn^w$ hypermutability. While the transposition of many prokaryotic transposable elements from a plasmid into the host chromosome has been demonstrated, such transpositions in a eukaryotic organism have not been previously described.

Although much remains to be learned about the mechanism by which transpositions occur, presumably the following events must take place. (i) The injected plasmid DNA is taken up into germ line cells; (ii) some of the injected sequences enter the nuclei of these cells where they are transcribed; (iii) element-coded RNA reaches the cytoplasm and is translated into one or more factors (transposase) required for transposition; (iv) transposase enters the nucleus where it catalyzes the insertion of one or more elements into the cellular chromosomes; (v) extrachromosomal copies of the injected DNA are eventually lost; (vi) inserted sequences are faithfully replicated and expressed in the injected organism and in those progeny that inherit them, giving rise to $sn^w$ mutability.

Our experiments demonstrate several interesting points concerning hybrid dysgenesis and P element function. P strains are characterized by the induction of hybrid dysgenesis when crossed to M strains. Hybrid dysgenesis is a syndrome of genetic traits, including sterility, the induction of mutations, $sn^w$ destabilization, chromosome rearrangements, male recombination, segregation distortion, and nondisjunction. It is likely that all these disparate effects result from the production of P element coded transposase in the germ line cells of dysgenic embryos and its subsequent action on the P elements resident in their genomes. The genetic determinants of hybrid dysgenesis, the P factors, are present in P strains at multiple chromosomal sites. Combinations of element-containing chromosomes from such strains are more effective in producing the dysgenic syndromes of sterility (*17*) or $sn^w$ hypermutability (*6*, *11*) than any single chromosome, suggesting that the number of active elements present in a dysgenic embryo may influence the frequency of P element transposition. Since lines containing a single P factor have never been described, it is not known whether the presence of a single P factor is sufficient to induce all the phenotypic manifestations of dysgenesis or whether some of them require the concerted action of multiple chromosomal P factors.

Our results indicate that the cloned 3-kb P element carried by pπ25.1 was itself sufficient to induce $sn^w$ mutability and P element transpositions. Thus, the 3-kb P element may be identical to the genetically defined P factor; all the information required for the hybrid dysgenesis syndrome may be contained on this element. We also have demonstrated that the presence of a single P element in the chromosomes of a strain is sufficient to cause it to behave as a P strain in its ability to induce $sn^w$ hypermutability. Whether strains bearing one or a few elements can attain the P cytotype remains to be tested.

These observations provide the basis for developing an efficient, controlled system of gene transfer in *Drosophila*. DNA segments of interest might be

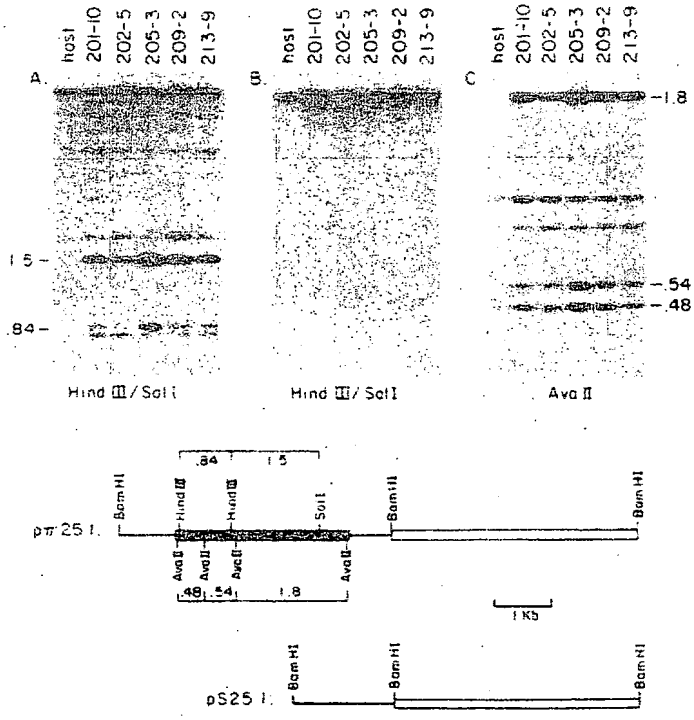


Fig. 6. Mutable lines contain complete P factors, but lack flanking and vector sequences from pπ25.1. DNA from the host $sn^w$ M strain and five independent mutable $sn^w$ G1 lines was digested with Hind III and Sal I (A and B) or with Ava II (C), subjected to electrophoresis and hybridized as in Fig. 3. The hybridization probe in (A) was the entire plasmid, pπ25.1; in (B), the entire pS25.1 plasmid; and in (C), the 0.84-kb Hind III and 1.5-kb Hind III–Sal I fragments of pπ25.1 were used. Linear maps representing the circular plasmids pπ25.1 and pS25.1 are shown. The solid bar indicates the position of the 3-kb P element in pπ25.1. The thin lines represent flanking *Drosophila* genomic DNA sequences from the cytogenic locus 17C and the open bar depicts pBR322 vector sequences. As shown in the diagram below, these probes differ only in the presence of a 3-kb P element in pπ25.1. Sequence analysis verified that pS25.1 would detect all sequences in pπ25.1 outside the 3-kb P element. The outermost Ava II sites within the 3-kb P element were shown to lie within the 31-bp inverted terminal repeats (9).

transposed into germ line chromosomes along with a P element into which the segment had been ligated in vitro. The accompanying article (*18*) demonstrates that highly efficient transfer of exogenous DNA's can indeed be accomplished with this approach.

### References and Notes


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5. The transpositional activity of P factors can be controlled with the rules of inheritance of cytotype [W. R. Engels, *Genet. Res.* 33, 137 (1979)]. Briefly, the P cytotype, which corresponds to the quiescent state of P elements, appears in strains where the genome contains many P factors. This state is thought to represent the presence of a P encoded repressor of transposition. Strains lacking P factors have the M cytotype—presumably the lack of this repressor. Once the P or M cytotype has been established, it tends to be maintained intact through the female line, thus showing partial independence of chromosomal constitution. Therefore, cytotype can be predicted for a particular individual by taking account of both the individual's genotype and its mother's cytotype.
6. Hypermutability of $sn^w$ requires, in addition to the M cytotype, the presence of P factors somewhere in the same cell. This additional requirement was seen when the $sn^w$ allele was isolated from its original P strain background (and the incidental chromosomal rearrangement surrounding the gene) by a series of recombination steps. The resulting strain (designated "$sn^w$ M") had no P derived material except in the immediate vicinity of the *singed* locus, and the $sn^w$ gene was observed to be entirely stable despite the M cytotype. However, it returned to its hypermutable condition whenever a P derived chromosome was crossed back into the genome. (The crosses had to be done in such a way as to preserve the M cytotype, of course.) Either major autosome or the X chromosome was sufficient to produce this effect, and only those chromosomes derived from P strains could function in this way. It was concluded that the element at $sn^w$ could be acted on by a *trans*-acting function provided by other P factors anywhere in the same genome, but that the $sn^w$ element could not produce this function itself. Furthermore, the $sn^w$ M strain stably maintained the M cytotype, implying that the $sn^w$ element did not have the cytotype-switching ability ascribed to other P factors (W. R. Engels, in preparation).
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9. K. O'Hare and G. M. Rubin, in preparation.
10. Techniques for microinjection of *Drosophila* embryos are discussed by E. B. Van Deusen [*J. Embryol. Exp. Morphol.* 37, 173 (1976)] and by S. Germeraad [*Nature (London)* 262, 229 (1976)]. A brief description follows. Supercoiled plasmid DNA's are prepared by ethidium bromide–CsCl gradient centrifugation. After removal of ethidium bromide (Dowex AG50W-X8 chromatography), the DNA is precipitated with ethanol and centrifuged. The pellet is washed several times with 70 percent ethanol in 0.2*M* NaCl and once with 70 percent ethanol. It is resuspended in injection buffer, which is 5 m*M* KCl and 0.1 m*M* $NaH_2PO_4$, *p*H 6.8. Injection needles are pulled from 25-μl Drummond Microcaps which have been siliconized prior to use. Low heat and high solenoid force are used to produce sharply tapered needles with tip diameters of less than 1 μm. Just prior to use, a needle is back-filled with DNA solution with the use of a 100-μl capillary pipette that has been drawn out in a flame. The DNA solution should be centrifuged (3 minutes in an Eppendorf centrifuge) just before loading the needle, to minimize clogging. Since we are using an air-filled injection system, air can remain on both sides of the DNA solution in the needle. The needle is mounted in the instrument holder and attached to the micromanipulator while embryos are prepared for injection. Embryos aged 0 to 90 minutes (that is, prior to pole cell formation) are used. Eggs are collected on lightly yeasted collection plates for 1 hour and then transferred to 18°C, the temperature used for subsequent steps. Eggs are gently transferred from the tray to a strip of double-stick tape on a microscope slide with a damp fly brush. Chorions are removed by lightly stroking the embryos with watchmaker's forceps under a dissecting microscope. After each shell is removed, the embryo is transferred to a small piece of double-stick tape on a cover slip (22 by 40 mm). Dechorionation and transfer of the embryos is facilitated by the use of a small ball of double-stick tape stickum held in the tip of the forceps. The embryos should be aligned on their sides with the posterior tip extending off the tape. Proper desiccation is determined empirically by the response of the embryos to injection. Excessive cytoplasmic leakage results from insufficient drying, while excessive desiccation results in a wrinkled embryo. Placement of a cover slip containing about 20 embryos (mounted over a 10-minute period) in a petri dish containing Drierite for 5 to 15 minutes is usually adequate. Desiccated embryos are immediately covered with halocarbon oil and mounted on the stage of an inverted microscope. A sharp point with a diameter of a few micrometers is obtained by lowering the needle into the oil and breaking off its extreme tip by running it into the double-stick tape while observing through the microscope. By means of a 10-ml syringe connected to the microinstrument holder and needle by air-filled plastic tubing, air is expelled from the needle until the DNA solution begins to flow out into the oil. The mounted embryos are injected by piercing the posterior end, drawing the needle tip as far back as possible while it is still inside the embryo, and expelling DNA solution. The DNA solution can be seen to enter the embryo. An amount appropriate to the level of desiccation of the embryo, about 1 to 5 percent of the egg volume, is expelled and the needle is rapidly withdrawn. After all of the embryos have been injected, damaged and improperly aged embryos are removed under a dissecting microscope and the cover slip is placed in a moist chamber. Hatched larvae are removed from the oil and transferred to standard food at 25°C.
11. The $sn^w$ allele arose in the progeny of a dysgenic hybrid [W. R. Engels, *Proc. Natl. Acad. Sci. U.S.A.* 76, 4011 (1979)]. In the M cytotype, $sn^w$ was found to mutate to two alternative states, $sn^c$ and $sn^+$ at total frequencies from 40 to 60 percent, depending on the particular sublines used. In the P cytotype, this allele became completely stable, suggesting that its hypermutability is controlled in the same way as the activities of P factors in general, and therefore that $sn^w$ itself might carry an inserted P element [W. R. Engels, *Genetics* 98, 565 (1981)]. Other studies revealed that $sn^w$ also served as a recurring breakpoint for dysgenesis-induced chromosome rearrangements [W. R. Engels and C. R. Preston, *Cell* 26, 421 (1981)], and its mutational changes were shown to be associated with the occurrence of lethal mutations elsewhere in the genome [J. D. Raymond and M. L. Simmons, *Genetics* 98, 291 (1981)]. Our hybridization (by Southern blot) studies support the previously postulated presence of one or more defective P elements at the *singed* locus in the $sn^w$ strain. The DNA from $sn^w$ strains that had mutated to $sn^c$ always showed an altered Hind III–Sal I fragment complementary to pπ25.1.
12. The observed lethality and sterility probably resulted from damage to the embryo caused by the injection procedure. The spectrum of visible defects observed was consistent with this interpretation. Most involved genital or abdominal structures: sterility usually resulted from an absence of germ line cells in the gonads. All these defects can result from abnormal development in the posterior region of the embryo—the site of injection. An embryo (volume: 2 nl) injected with an estimated 40 pl of DNA solution would therefore receive about $4 \times 10^{-6}$ μg of plasmid at the optimum concentration, corresponding to $4 \times 10^5$ molecules, or an amount of DNA equivalent to that present in about ten embryonic cells. The reason for the decreased rate of mutability at high DNA concentrations is unknown. One possibility is that the large number of P element termini compete with the termini of the integrated defective elements at $sn^w$ for binding of a limited amount of transposase. Alternatively, large numbers of injected P elements might produce sufficient repressor to change the cytotype of the host embryo to P.
13. The correlation between mutability and the presence of complete P element sequences was verified in seven of these eight cases. Several progeny from embryo 102 contained complete P element sequences by the above test, but did not show mutability. These flies displayed an unusual *singed* phenotype intermediate between $sn^w$ and wild type. The lack of mutability in this one case is probably due to the loss or rearrangement of one of the defective P elements located at the *sn* locus, which results in both the altered phenotype and the loss of hypermutability. A less likely alternative is that the P element in this strain could have been defective, despite the presence of intact 0.84-kb and 1.5-kb internal fragments.
14. For an explanation of cytogenetic nomenclature see C. B. Bridges [*J. Hered.* 26, 60 (1935)].
15. The chromosomal locations of P factors in several G2 lines derived from a single injected embryo were compared. In some cases the sites of pπ25.1 hybridization in such sibling strains contained one or more sites in common, as expected for premeiotic transposition events followed by normal meiotic segregation. In other cases sites did not overlap but, since only two to three larvae from each line were analyzed, autosomal inserts present on only one homolog may not have been detected. Both the $sn^c$ line 301-1 and the $sn^+$ line 301-3 contained the 12F insert, demonstrating that the $sn^w$ mutation could occur subsequent to P element integration. The analyses were limited by our ignorance of the timing and frequency of P element transposition in the injected strains. It will be important to determine: (i) How many independent transpositions from plasmid to chromosome occur in the germ cells of an injected embryo? (ii) To what extent is site multiplicity the result of the secondary transposition of chromosomally integrated elements? That secondary transposition can occur was demonstrated by the detection of a P factor in the attached-X chromosome used to balance one of the transformed lines. This attached-X chromosome was not present in the injected embryo. (iii) How many pole cells initially take up and integrate P element DNA? (iv) To what extent is $sn^w$ mutability correlated with P element transposition?
16. Genetic data suggest that P factors insert more frequently at some sites than others [M. J. Simmons and J. K. Lim, *Proc. Natl. Acad. Sci. U.S.A.* 77, 6042 (1980)]. In our experiments, P element inserts at the sites of the two major "hot spots" of hybrid dysgenesis-induced mutations, *singed* and *Beadex*, could not be detected unambiguously, because of the presence of sequences homologous to the pπ25.1 probe near *singed* (7D) and *Beadex* (17C) in the $sn^w$ host strain. The distribution of sites in Fig. 5, while diverse, may not be random as evidenced by the clustering of sites in the proximal part of the X chromosome, for example.
17. W. R. Engels, *Genet. Res.* 33, 219 (1979).
18. G. M. Rubin and A. C. Spradling, *Science* 218, 348 (1982).
19. Occasional chromosome rearrangements were observed in salivary gland chromosomes from the G1 lines, but were not detected in the host strain. Rearrangements could have been induced in the germ line cells of the injected embryos or might have occurred in later generations in lines carrying intact P elements. Since detailed analysis and comparison between sibling G1 lines was not carried out, it is not known whether lines carrying one or a few P elements underwent frequent chromosome rearrangements. During normal dysgenesis, chromosome rearrangements take place preferentially at the sites of resident P factors [W. R. Engels and C. R. Preston, in (*11*)]. Furthermore, the 3-kb P element cloned in pπ25.1 is derived from cytogenetic locus 17C in the $\pi_2$ strain. Chromosome rearrangements with breakpoints at this site are observed at high frequency in this strain, suggesting that the sequences in pπ25.1 may be competent to induce rearrangements in vivo. No association of chromosome rearrangement breakpoints and P factor sequences was observed in these experiments, however.
20. The $sn^+$ phenotype observed in many of the $sn^w$ derivatives could be separated from true wild type since in a population of such $sn^+$ flies a fly containing a single bristle with an acute bend (usually one of the scutellars) would frequently be observed.
21. A. C. Spradling, *Cell* 27, 193 (1981).


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